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Broad-Spectrum Antiviral Activity of 2-β-D-Ribofuranosylselenazole-4-Carboxamide, a New Antiviral Agent†

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The relative in vitro antiviral activities of three related nucleoside carboxamides, ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide), and selenazole (2-β-D-ribofuranosylselenazole-4-carboxamide), were studied against selected DNA and RNA viruses. Although the activity of selenazole against different viruses varied, it was significantly more potent than ribavirin and tiazofurin against all tested representatives of the families Paramyxoviridae (parainfluenza virus type 3, mumps virus, measles virus), Reoviridae (reovirus type 3), Poxviridae (vaccinia virus), Herpesviridae (herpes simplex virus types 1 and 2), Togaviridae (Venezuelan equine encephalomyelitis virus, yellow fever virus, Japanese encephalitis virus), Bunyaviridae (Rift Valley fever virus, sandfly fever virus [strain Sicilian], Korean hemorrhagic fever virus), Arenaviridae (Pichinde virus), Picornaviridae (coxsackieviruses B1 and B4, echovirus type 6, encephalomyocarditis virus), Adenoviridae (adenovirus type 2), and Rhabdoviridae (vesicular stomatitis virus). The antiviral activity of selenazole was also cell line dependent, being greatest in HeLa, Vero-76, and Vero E6 cells. Selenazole was relatively nontoxic for Vero, Vero-76, Vero E6, and HeLa cells at concentrations of up to 1,000 μg/ml. The relative plating efficiency at that concentration was over 90%. The effects of selenazole on viral replication were greatest when this agent was present at the time of viral infection. The removal of selenazole from the medium of infected cells did not reverse the antiviral effect against vaccinia virus, but there was a gradual resumption of viral replication in cells infected with parainfluenza type 3 or herpes simplex virus type 1 (strain KOS). However, the antiviral activity of ribavirin against the same viruses was reversible when the drug was removed.

A novel selenazole carboxamide nucleoside, *3* (2-β-D-ribofuranosylselenazole-4-carboxamide) (Fig. 1), has recently been synthesized in our laboratory (34). Its antiviral activity has not been previously evaluated, although our preliminary studies indicated that selenazole *3* possesses significant antitumor effects (34). Selenazole was designed as an analog of tiazofurin *2* (2-β-D-ribofuranosylthiazole-4-carboxamide). Preliminary studies of tiazofurin *2* (33) indicated that it possesses a spectrum of antiviral activity in cell culture similar to, but somewhat less potent than, that of ribavirin *1* (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) and that it has a significant activity against several experimental neoplasms, including those of the lung (15, 18, 26).

Ribavirin, a related ribofuranosylcarboxamide, has a broad spectrum of antiviral activity (32), both in vitro (2, 14, 20, 24) and in vivo (13, 16, 19, 31, 37), and is currently under clinical evaluation for safety and efficacy in humans. Recent studies have shown that ribavirin is remarkably effective clinically against influenza A and B (17, 21), hepatitis (23), and respiratory syncytial virus infection (11). These recent studies in humans have renewed considerable interest in the potential therapeutic utility of ribavirin (7).

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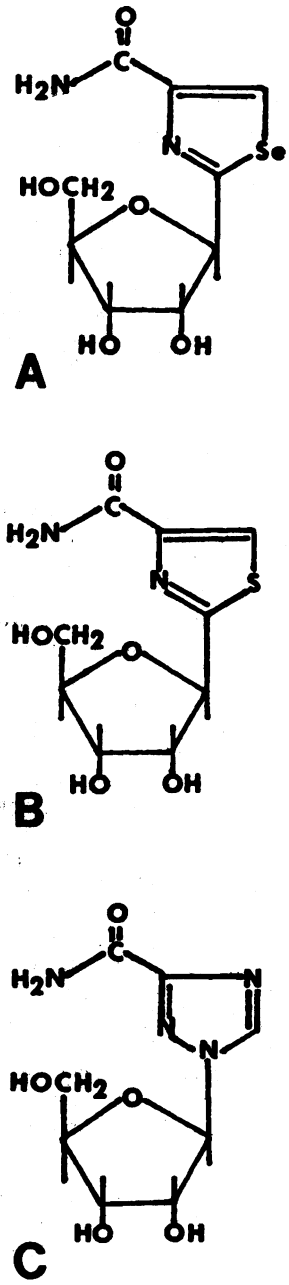

FIG. 1. Chemical structures of selenazole *3* (A), tiazofurin *2* (B), and ribavirin *1* (C), the three related nucleoside carboxamides used in this study.

In view of the close structural relationship of nucleosides *2* and *3* to ribavirin *1*, we compared the in vitro antiviral activities of these nucleosides against a number of DNA and RNA viruses. In the present in vitro study, we report that selenazole *3* appears to have superior in vitro antiviral activity as compared with those of ribavirin and tiazofurin.

MATERIALS AND METHODS

Cells. Five types of continuous cell lines were used in this study: three strains of African green monkey kidney cells (Vero, ATCC CCL 1; Vero #6, ATCC CRL 1586; Vero-76, ATCC CRL 1587) and one strain of rhesus monkey kidney cells (LLC-MK_2, ATCC CCL 7; American Type Culture Collection Cell Repository, Rockville, Md. and human epithelioid cervical carcinoma cells (HeLa; Flow Laboratories, Ingelwood, Calif.). Cells were grown in antibiotic-free Eagle minimum essential medium (EMEM) with Earle salts supplemented with 10% newborn bovine serum (GIBCO Laboratories, Grand Island, N.Y.), except for Vero-76 cells which were grown in EMEM with Earle salts and 1× nonessential amino acids supplemented with 10% fetal bovine serum (FBS; KC Biologicals Inc., Lenexa, Kans). LLC-MK_2 cells were grown in medium 199 with Earle salts supplemented with 10% FBS. All cells were passaged in 75-cm^3 plastic flasks (Corning Glass Works, Corning, N.Y.) at 37°C under humidified 5% CO_2–95% air. All of the experiments reported in this paper were conducted with cells which had been passaged between 5 and 30 times in our laboratory. All cell cultures were periodically tested and found to be free of bacterial and mycoplasmal contamination.

Viruses. Twenty viruses, representing the major families of DNA and RNA viruses pathogenic for humans, were used in these experiments. Herpes simplex virus type 1 (HSV-1) strain KOS was supplied by Byron K. Murray, Virginia Commonwealth University, Richmond, Va. Herpes simplex virus type 2 (HSV-2) strain MS, vaccinia virus (VV) strain Elstree, adenovirus type 2 (Ad2) strain Adenoid 6, coxsackievirus B1 strain Ha 201468, coxsackievirus B4 strain J.V.B. (Benschoten), echovirus type 6 strain Damori, mumps virus strain Enders, measles virus strain Edmonston, reovirus type 3 strain Abney, and encephalomyocarditis virus strain EMC were obtained from the American Type Culture Collection. Vesicular stomatitis virus (VSV) strain Indiana was acquired from Ron W. Leavitt, Brigham Young University, Provo, Utah. Parainfluenza virus type 3 (Para-3) strain C243 was donated by R. W. Sidwell, Utah State University, Logan.

Virus pools were prepared by infecting confluent monolayers of HeLa or Vero cells. Infected monolayers were incubated for 2 to 6 days at 37°C in 5% CO_2 and then harvested when cultures exhibited 90% cytopathic effect (CPE). Viruses were passaged at least twice in each cell line before the virus pool was used. The cell line used in any given antiviral experiment was infected with virus produced only in that same cell line. Infected cells and fluids were frozen at −70°C. Intracellular virus was released by disrupting infected cells by freeze-thawing and vigorous trituration against the flask wall. Viral stock cultures were divided into 0.5-ml samples, frozen, and stored at −70°C until used. Samples of each virus were assayed for infectious virus by a plaque formation assay (PFU per milliliter) and by determination of the 50% tissue culture infective dose by the method of Reed and Muench (25).

Rift Valley fever virus, zagazig 501 strain (8), was passaged once in Vero-76 cells. Venezuelan equine encephalomyelitis virus (VEE), Trinidad donkey

strain (12), was passaged once in Peking duck primary cells. Pichinde virus, CoAn 3739, ATCC VR-708, was passaged once in Vero-76 cells. Yellow fever virus (YF), Asibi strain, was obtained as plasma from a single infected rhesus monkey (*Macaca mulatta*) and plaqued on LLC-MK_2 cells without adaptation in tissue culture (35). Japanese encephalitis virus (JE), strain Nakayama, was obtained at suckling mouse passage 54 from Robert Shope, Yale Arbovirus Research Unit, New Haven, Conn., and passaged one additional time in Vero-76 cells. Sandfly fever virus (Sicilian), Brownell strain, was from a human isolate (1). Hantaan virus, the etiological agent of Korean hemorrhagic fever, strain HBL7990, was isolated by Ho Wang Lee, Institute for Viral Diseases, Korea University, Seoul, from human material and passaged 1 time in *Apodemus agarius corae* and 16 times in Vero E6 cells. All studies involving Rift Valley fever virus, sandfly fever virus (Sicilian), YF, JE, VEE, Pichinde virus, and Korean hemorrhagic fever virus were performed in compliance with guidelines (4, 36) in a closed suite, utilizing facilities designed for high-level microbiological containment (class 3) at the U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Md.

Antiviral compounds. Ribavirin, tiazofurin, and selenazole compounds were synthesized and prepared in our laboratory as previously described (33, 34, 37). A 10^{-2} M stock solution of each compound was prepared in deionized distilled water. The pH was adjusted to 7.35 with 0.1 N sterile NaOH. The stock solution was sterilized by passage through a 0.22-μm membrane filter (Gelman Sciences, Inc., Ann Arbor, Mich.). For the viruses listed in Table 3, drugs were prepared as described below and adjusted to pH 7.3.

Determination of VR. Vero or HeLa cells were inoculated into 96-well culture plates (Corning) at a concentration of 5×10^4 cells per 0.2 ml per well and cultured for 24 h at 37°C in 5% CO_2 to confluency. Cells in the plates were infected with a proper predetermined 50% tissue culture infective dose of virus (0.1 ml per well). Virus was allowed to adsorb for 30 min at 37°C in 5% CO_2. After adsorption, the test compounds were added in seven 0.5-$\log_{10}$ dilutions ranging from 5×10^{-3} to 5×10^{-6} M in 0.1 ml of diluent. At each dilution, duplicate wells were used for evaluation of antiviral activity, and single wells were used for determination of cytotoxicity.

The degree of inhibition of virus-induced CPE and drug cytotoxicity was observed microscopically after 72 h of incubation. CPE was scored numerically from 0 (normal control cells) to 4 (100% cell destruction as in virus controls) to calculate a virus rating (VR) as previously reported (30).

Determination of drug ED_{50}. The 50% effective dose (ED_{50}) values were scored microscopically during VR readings and double-checked from the crystal-violet-stained 96-well plates. In several cases, the ED_{50} values were also confirmed by the following plaque reduction method.

Vero or HeLa cells were seeded into 24-well culture plates (Corning) at a concentration of 2×10^5 cells per ml per well and grown for 24 h at 37°C in 5% CO_2. The plates were infected with 100 to 150 PFU of virus in 0.2 ml and incubated for 30 min at 37°C in 5% CO_2 to allow adsorption of virus. The test compounds were then added to duplicate wells in seven 0.5-$\log_{10}$ dilutions (0.1 ml per well), and the cells were overlaid with 1% methylcellulose in EMEM with 2% newborn calf serum (1.0 ml per well). The plates were incubated for 2 to 3 days to allow for plaque formation. Cells were then fixed with 10% Formalin and stained with 1% crystal violet in water. Plaques were counted, and the ED_{50} was extrapolated graphically from the plotted values. The concentration of compound that resulted in a 50% reduction of plaque formation, as compared with nondrug controls, was designated the ED_{50}.

YF was assayed as described by Canonico et al. (3); Rift Valley fever virus, sandfly fever virus (Sicilian), VEE, JE, and Pichinde virus were assayed in triplicate in Linbro six-well plates (9.6 cm^2 per well; Flow Laboratories) in confluent monolayers of Vero-76 cells. The compounds were tested using a threefold dilution series of the drug prepared in diluent medium consisting of EMEM-nonessential amino acids, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 10 μg of gentamicin per ml, and 5% (vol/vol) heat-inactivated FBS. Cells were preincubated with 0.5 ml of drug for 4 h at 37°C in 5% CO_2, and 100 PFU of virus in the appropriate dilution of drug was adsorbed in 0.2 ml at 37°C in 5% CO_2 with shaking every 15 min for a total of 60 min. The plates were then overlaid with Eagle basal medium with Earle salts (without phenol red)–17 mM HEPES–5% heat-inactivated FBS–10 μg of gentamicin per ml in 0.5% agarose containing the appropriate concentration of drug. Plates were then incubated at 37°C in 5% CO_2 for the appropriate time, and the plaques were visualized after staining with neutral red (final concentration, 1:6,000) diluted in Puck saline A containing 5% (vol/vol) heat-inactivated FBS and 10 μg of gentamicin per ml. The plaques were counted after an additional incubation period of 13 to 24 h. Korean hemorrhagic fever virus was assayed by a modification of the plaque assay described by McCormick et al. (22), using Linbro 12-well plates containing confluent monolayers of Vero E6 cells. Hantaan virus (50 PFU in 0.1 ml of diluent) was adsorbed at 38°C in 5% CO_2 with shaking every 15 min for 60 min. The plates were then overlaid with 0.9 ml of overlay medium (EMEM [without phenol red], 2× nonessential amino acids, 8 mM glutamine, 10% (vol/vol) heat-inactivated FBS, 10 μg of gentamicin per ml, 0.6% SeaKem ME agarose) containing the appropriate concentration of drug. Plates were then incubated at 38°C in 5% CO_2 for 6 days. The plaques were visualized after staining with a solid neutral red overlay (final concentration, 1:6,000) diluted in overlay medium. The ED_{50} was calculated using a four-parameter logistic curve fitting computer program as described by Rodbard and Hutt (27).

Concentration, time, and MOI studies. The quantitation of total virus yield was performed on confluent Falcon 24-well plates seeded with Vero cells, using a routine plaque reduction assay (28). Low multiplicity of infections (MOIs) were used to minimize the production of defective interfering particles during incubation.

Cellular cytotoxicity. HeLa and Vero cells were used for normal cytotoxicity studies. These cells were grown overnight on Linbro 96-well plates until near confluency. The medium was then replaced with growth medium containing one of the three test compounds at a 100- or 1,000-μg/ml concentration. Quadruplet wells were used for each drug concentration,

TABLE 1. Comparative antiviral activities of selenazole, tiazofurin, and ribavirin

Virus[a]	VR[b]					
	Vero cells			HeLa cells		
	Selenazole	Tiazofurin	Ribavirin	Selenazole	Tiazofurin	Ribavirin
RNA						
Para-3	2.6	1.3	1.3	2.2	1.8	1.6
Measles	2.0	1.1	1.1	1.8	1.4	1.1
Mumps	1.7	0.9	1.0	1.0	1.6	0.7
Reo 3	1.8	1.6	1.6	2.7	1.9	2.0
VSV	0.4	0.5	0.6	2.1	1.1	1.3
Coxsackie B1	1.7	0.9	0.9	1.8	1.0	1.4
Coxsackie B4	0.0	0.0	0.5	2.2	1.3	1.5
Echo 6	0.5	0.2	0.3	0.7	0.5	0.6
EMC[c]	—[d]	—	—	2.0	1.5	1.6
DNA						
VV	2.1	1.4	1.5	2.4	1.3	1.5
Ad2	—	—	—	1.9	1.0	1.3
HSV-1	1.2	0.9	0.8	1.4	0.5	0.3
HSV-2	1.5	0.9	0.7	1.5	1.6	0.9

[a] A complete description of viruses is given in the text.
[b] Determined as described in the text. Values represent averages of 3 to 12 determinations, depending on the drug, virus, and cell line used.
[c] EMC, Encephalomyocarditis virus.
[d] —, No data available.

along with an equal number of control (nondrug) wells. Cells were exposed to the drug from 24 to 72 h, after which the cells were harvested by trypsinization, triturated, and serially diluted, and 1/10 dilutions were plated onto Costar 12-well plates. After 3 to 5 days of incubation in drug-free growth medium, the cells were washed with phosphate-buffered saline, fixed with 10% Formalin, and stained with crystal violet. Cell colonies, as representatives of single viable original cells, were counted under a dissecting microscope. Relative plating efficiency, as percent survival, was calculated as the average number of colonies grown from drug-treated cells divided by the average number of control (untreated) cells grown under similar growth conditions.

RESULTS

Antiviral activity. The in vitro effects of selenazole *3*, tiazofurin *2*, and ribavirin *1* against selected representatives of both DNA and RNA virus families are summarized in Tables 1, 2, and 3. The overall in vitro effectiveness of the drugs are expressed in VR values, and the quantitative potency of the antiviral agent is evaluated from its ED_{50} values. Regardless of the methods used for evaluation, selenazole appeared to be the most effective antiviral compound.

Of the DNA viruses studied, VV, HSV-1, and HSV-2 were inhibited most by selenazole. The adenoviruses have been shown to be quite insensitive to the effects of most antiviral compounds (26). However, in this study, Ad2 was significantly inhibited in HeLa cells by selenazole.

Among the RNA viruses tested, representatives of the families Myxoviridae, Reoviridae, Togaviridae, Bunyaviridae, and Arenaviridae were inhibited most effectively by selenazole. The representatives of the family Picronaviridae as well as of the family Rhabdoviridae were effectively inhibited in HeLa cells but not in Vero cells.

Concentration effect of selenazole on virus replication. The methods of assay utilized in Tables 1 and 2, i.e., VR and ED_{50}, do not allow one to determine whether the viral inhibition by selenazole was due to the prevention of viral maturation or due to a masking effect of cellular CPE by the drug. Therefore, the inhibition of the production of infectious virus by various amounts of selenazole was determined in Vero cells infected with four different viruses: HSV-1, Para-3, VV, and VSV. Data presented in Fig. 2 show a concentration-dependent inhibition of infectious virus production by selenazole of HSV-1, VV, and Para-3. However, the data indicate no inhibition of VSV replication in Vero cells by this drug.

The concentration effect of selenazole was linear and broad against VV and HSV-1 (KOS) over a 3-$\log_{10}$ dilution but very abrupt and narrow with Para-3. This seems to reflect the observed CPE in Vero cells. Against Para-3, an almost all-or-nothing response was seen; however, against VV and HSV-1 (KOS), diminishing numbers and sizes of plaques with higher selenazole concentrations were observed.

TABLE 2. Comparative antiviral activities of selenazole, tiazofurin, and ribavirin

Virus[a]	ED_{50} (μg/ml)[b]					
	Vero cells			HeLa cells		
	Selenazole	Tiazofurin	Ribavirin	Selenazole	Tiazofurin	Ribavirin
RNA						
Para-3	1	150	38	1	11	70
Measles	1	42	7	2	86	14
Mumps	5	42	40	1	3	7
Reo 3	1	9	2	8	3	1
VSV	1,000	1,000	1,000	9	200	20
Coxsackie B1	15	130	100	10	130	35
Coxsackie B4	1,000	1,000	700	6	86	36
Echo 6	1,000	1,000	1,000	51	130	106
EMC[c]	—[d]	—	—	5	43	22
DNA						
VV	3	45	18	2	60	16
Ad2	—	—	—	9	140	20
HSV-1	30	100	95	2	130	—
HSV-2	10	160	80	4	4	100

[a] A complete description of viruses is given in the text.
[b] ED_{50} Determined as described in the text. Values represent averages of 3 to 12 determinations, depending on the drug, virus, and cell line used. The highest concentration tested was 1,000 μg/ml.
[c] EMC, Encephalomyocarditis virus.
[d] —, No data available.

Effect of the time of selenazole addition on the inhibition of viral replication. In the studies described above, selenazole was added immediately after viral adsorption, and the cells were incubated in the presence of the drug until the cultures were assayed. To elucidate the in vitro time dependency of the antiviral activity of selenazole, the effects of adding selenazole before and after viral infection were evaluated in Vero cells. In addition, the reversibility of the antiviral activity of selenazole was studied by removing the drug at various times after viral infection (Table 4).

The data indicate that when selenazole was added up to 24 h before infection of Vero cells, no significant time dependency existed against VV and Para-3; both were inhibited during any exposure time by over 99%. However, HSV-1 (KOS) seemed to show a moderate time dependency to selenazole under these test conditions. Under similar growth conditions, with a low MOI (0.004) and with virus incubated in the presence of selenazole at a concentration of 10^{-5} M, the simultaneous addition of selenazole and virus caused only about 60% HSV-1 inhibition. Para-3 was inhibited by 100%, and VV was inhibited by 95% (Fig. 2). When added up to 8 h postinfection, selenazole still showed over 99% inhibition against VV and Para-3 and 60% inhibition against HSV-1 (KOS). The removal of selenazole after 4 and 8 h postinfection caused a moderate loss of its antiviral activity against HSV-1 and Para-3 but not against VV. Again, replication of VSV in Vero cells was not affected by selenazole. In general, selenazole has a much better antiviral activity than does ribavirin as measured with the parameters calculated in Table 4. The potent antiviral effect of ribavirin against VV (Table 4) has been proposed by

TABLE 3. ED_{50} of selenazole, tiazofurin, and ribavirin against selected arboviruses and arenaviruses

Virus[a]	ED_{50} (μg/ml)[b]		
	Selenazole	Tiazofurin	Ribavirin
Alphavirus (VEE)	6.7	NA[c]	190.0
Flavivirus			
YF	0.005	9.2	46.4
JE	3.0	73.0	42.0
Bunyavirus[d]			
RVF	2.8	NA	80.0
SFS	1.2	3,253.2	77.2
KHF	1.6	1.5	14.8
Arenavirus (Pichinde)	4.0	494.5	61.0

[a] A complete description of viruses is given in the text.
[b] Determined as described in the text.
[c] NA, No activity observed at 5,200 μg/ml.
[d] RVF, Rift Valley fever virus; SFS, sandfly fever virus (Sicilian); KHF, Korean hemorrhagic fever virus.

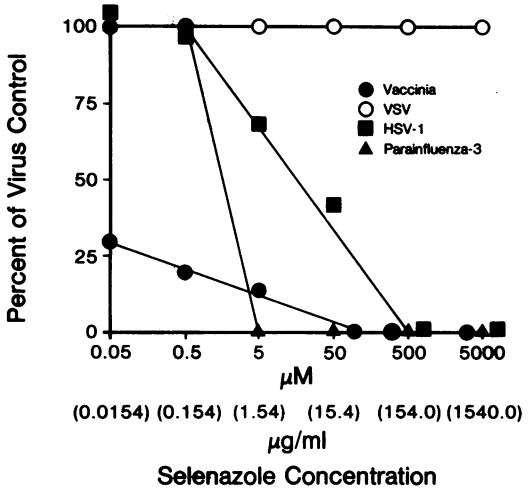

FIG. 2. Effect of different concentrations of selenazole on virus replication. Vero cells were infected with virus at MOIs of 0.04 to 0.05. Selenazole was added immediately after infection. Total infectious virus was determined after 72 h and compared with non-drug-treated controls.

several investigators (10, 29) to be due to the inhibition of the specific capping mechanism of its mRNA.

Effect of MOI on antiviral activity. Vero cells were infected at various MOIs (0.007 to 5) with HSV-1, Para-3, VV, and VSV. Figure 3 shows the results of the inhibition of total virus yield in the presence of a constant (10^{-5} M) amount of the drug and increasing amounts of the appropriate virus. Selenazole was added immediately after virus infection.

The data demonstrate that as the MOI was increased, the antiviral effect of selenazole was decreased. With HSV-1, the loss of antiviral activity after initial inhibition diminished gradually in a linear manner with the increasing virus dose. This might indicate a virustatic state, reflecting a continuous need for the drug to exert its antiviral activity. However, Para-3 and VV showed a completely different dose response. The antiviral state was effective over a wide range of MOI (5 to 500 50% infective doses) but was then abruptly overcome. No antiviral effect was seen with VSV, implying a complete insensitivity of this virus to selenazole in Vero cells.

Cellular cytotoxicity. In preliminary Vero toxicity studies, no cytotoxicity would be detected by microscopic examination or by utilization of staining techniques in HeLa, Vero, Vero-76, LLC-MK_2, and Vero E6 cells when they were treated with 1,000-μg/ml quantities of the three test compounds.

The relative plating efficiency in Vero and HeLa cells after 30 h of exposure was over 95% at 100 μg/ml and over 90% at 1,000 μg/ml. The relative plating efficiency of selenazole after 72 h of exposure in Vero cells at 100 μg/ml was still over 95%. However, at 1,000 μg/ml, the relative

TABLE 4. Effect of the time of addition of drug on the reduction in virus titer in Vero cells

Drug addition[a] (± h)	Reduction in virus titer[b] (%) at 72 h							
	Para-3[c]		HSV-1		VV		VSV	
	Selena[d]	Riba[d]	Selena	Riba	Selena	Riba	Selena	Riba
Pretreatment								
(−) 24–72	>99	23	99	0	>99	88	0	0
(−) 8–72	>99	0	90	74	>99	47	0	0
(−) 4–72	>99	0	90	70	>99	89	0	0
Simultaneous (0–72)	>99	0	89	58	>99	93	0	0
Postinfection								
(+) 4–72	>99	0	92	82	>99	93	0	0
(+) 8–72	>99	0	60	41	>99	92	0	0
Reversal[e]								
(*) 4–28	80	0	65	0	99	80	0	0
(*) 8–32	59	0	57	29	95	98	0	0

[a] Selenazole and ribavirin were added to cultures of Vero cells at 10^{-5} M. −, Drug addition before viral infection; +, drug addition after viral infection.

[b] Titers of samples were determined for total infectious virus 72 h after viral inoculation of Vero cells; data were compared with non-drug treated controls and expressed as percent reduction in virus titer.

[c] Cultures were infected at MOIs of 0.004 to 0.005 with Para-3, HSV-1, and VV. VSV was used at an MOI of 0.007.

[d] Selena, Selenazole; Riba, ribavirin.

[e] Drug was removed after (*) hours of viral infection by washing the cells three times with phosphate-buffered saline solution (pH 7.2); the drug-free cells then were incubated for an additional 24 h before virus titration.

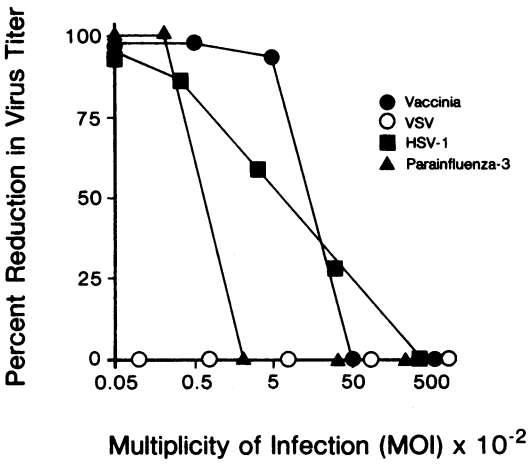

FIG. 3. Effect of MOI on inhibition of virus replication by selenazole. Vero cells were infected at various MOIs and treated with 10^{-5} M selenazole. Total infectious virus was determined after 72 h.

plating efficiency of Vero cells was diminished to 70%, demonstrating a mild cytotoxicity at this high concentration over the longer exposure time.

DISCUSSION

The experiments in this study were designed so that all three nucleosides were tested in the same microplate against one virus in a single cell line. Thus, for a given experiment, the following parameters were constant: (i) the number of times the cells were passaged, (ii) the drug lot and dilution used, (iii) the conditions under which the virus pool was frozen and thawed, and (iv) the amount of virus (50% tissue culture infective dose units) added to each microplate well.

Our results indicate that selenazole has a broad-spectrum antiviral activity against both DNA and RNA viruses. Of the DNA viruses studied, the representatives of the families Poxviridae (VV) and Herpesviridae (HSV-1, HSV-2) were inhibited most, but the greatest antiviral activity of selenazole was observed against the representatives of RNA viruses, particularly the families Paramyxoviridae (Para-3, mumps virus, measles virus), Reoviridae (reovirus type 3), Togaviridae (VEE, YF, JE), Bunyaviridae (Rift Valley fever virus, sandfly fever virus [Sicilian], Korean hemorrhagic fever virus), and Arenaviridae (Pichinde virus). Good, although cell-dependent, antiviral activity was measured in the families Adenoviridae (Ad2), Picornaviridae (coxsackieviruses B1 and B4), and Rhabdoviridae (VSV).

Selenazole has been observed to affect the reproduction of both DNA and RNA viruses in several parameters. The plaque number, plaque size, and titer of released virus, as well as intracellular virus production, were all reduced. This antiviral activity seems to be cell-line dependent, being most effective in HeLa cells. The phenomenon that a compound may be relatively ineffective against a particular virus in one cell line but exhibit high activity in another is a common observation (5, 32).

These preliminary studies also may indicate that the antiviral activity of selenazole is both virucidal (against VV) and virustatic (against Para-3 and HSV-1), depending upon the virus and cell line used. Also, the pretreatment use of selenazole seems to enhance its antiviral activity against virustatic species, such as HSV-1 (Tables 1 and 2; Fig. 3).

The activity of an antiviral agent in cell culture depends on several biological processes: (i) cell membrane permeability, (ii) intracellular conversion of the drug to its active or inactive form by cellular enzymes, and (iii) selective recognition of the drug by virus-induced or cell enzymes (6, 9, 38). The slightly lower VR values in Vero cells against adenoviruses and picornaviruses do not seem to be caused by the inability of selenazole to penetrate the cell membrane, since other viruses are effectively inhibited in these cells. More fundamental biochemical processes relating to the permissiveness or nonpermissiveness of the cell-virus assay systems seem to be more likely causes for these variations.

Selenazole is nontoxic to Vero, Vero-76, Vero E6, LLC-MK_2, and HeLa cells in 1,000-μg/ml quantities (highest concentration tested). The lack of cytotoxicity was confirmed by microscopic observation and crystal violet or neutral red staining procedures during VR and ED_{50} assay readings. However, when cytotoxicity was measured with relative plating efficiency, about 30% of the cells were not able to divide after 3 days of exposure with 1,000 μg of selenazole per ml.

Although tiazofurin and ribavirin show relatively equal antiviral potency, the antiviral activity of selenazole was noticeably better than either agent against all viruses tested in this study. However, all three agents possess a somewhat similar antiviral spectrum. It appears that selenazole exerts a greater antiviral inhibitory effect over a longer period of time than does ribavirin (Table 4).

These preliminary findings and the marked antiviral effects of selenazole warrant a further evaluation of its efficacy in other cell-virus assay systems and its potential in vivo usefulness. Further studies are planned to elucidate the antiviral mechanism of selenazole in different cell-virus assay systems and animal virus models.

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